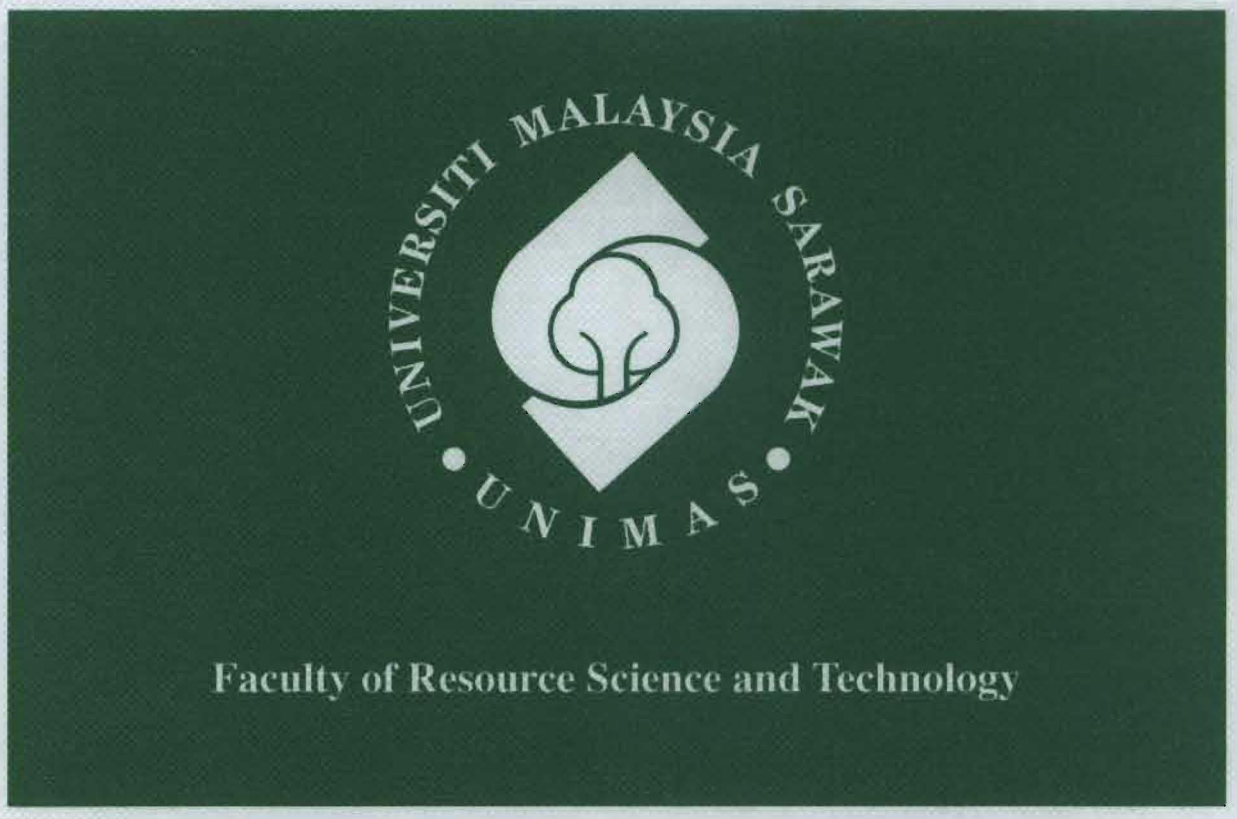

EFFECTS OF CASTOR OIL ON *ESCHERICHIA COLI* K011 GROWTH AND FERMENTING ACTIVITIES DURING ANAEROBIC FERMENTATION

Ennry Anak Esut

Bachelor of Science with Honours
(Resource Biotechnology)
2012

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DECLARATION

I hereby declare that this Final Year Project report 2012 entitled "Effects of Castor Oil on *Escherichia coli* K011 Growth and Fermentation Activities during Anaerobic Fermentation" is based on my original work except for the quotations and citations which have been dully acknowledged also, declare that it has not been or concurrently submitted for any other degree at UNIMAS or other institutions of higher learning.

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LIST OF ABBREVIATIONS

HPLC	High Performance Liquid Chromatography
%	Percentage
°C	Degree celcius
G	Gram
µl	Microliter
mL	Milliliter
rpm	Revolutions per minute
spp.	Species
E. coli K011	*Escherichia coli* K011
wt	Weight
g/L	Gram per liter
CFU	Colony Forming Unit

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Effects of Castor Oil on *Escherichia coli* K011 Growth and Fermentation Activities during Anaerobic Fermentation

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ABSTRACT

Bioethanol is one of the biofuel that is widely studied presently. This is due to the decreasing of fossil fuel. Researchers are making various efforts to find other fuel alternative to resolve this issue. One way is the production of bioethanol from lignocellulosic waste. Thus, there is a very big opportunity in developing fuel ethanol from agricultural residue and other lignocellulosic feedstock because it is low in cost. Bioethanol productions majorly used are fermentation technique. One of major problem in fermentation is ethanol accumulation. Therefore with existence of castor oil this problem can be solved. Ability of castor oil in absorption of ethanol gives huge impact in the fermentation process. In this project *Escherichia coli* K011 is main fermenting agent toward glucose. But from the result existence of castor oil in fermentation broth give some effects toward the growth and fermenting activities. There are better results obtained from experiment conducted without castor oil. This could be due to several factors. Firstly toxicity character that castor oil has affected *E. coli* K011 activities. Then, another factor is glucose is not the best substrate for *E. coli* K011 to consume. Therefore, for better production of ethanol for *E. coli* K011 xylose is more preferred.

Keywords: *Bioethanol, fossil fuel, lignocellulosic waste, fermentation, castor oil, Escherichia coli K011, glucose, xylose.*

ABSTRAK

Bioetanol merupakan salah satu penghasilan minyak secara biologi yang banyak dipelajari pada hari ini. Ini adalah kerana pengurangan minyak mentah yang boleh didapati dari dalam bumi. Penyelidik banyak mencari cara lain untuk penghasilan minyak bagi menyelesaikan isu pengurangan minyak ini. Salah satu caranya adalah mengunakan bahan buangan daripada tumbuhan. Peluang yang besar boleh didapati daripada bidang ini kerana penghasilan minyak boleh dilakukan dengan kadar kos yang rendah. Bioetanol biasanya dihasilkan daripada proses fermentasi. Akan tetapi dalam proses fermentasi ini satu maslah utama yang sering dihadapi adalah pengumpulan atau permendakan ethanol iaitu hasil fermentasi. Permendakan ini boleh diatasi dengan kehadiran minyak kastor didalam sistem fermentasi itu sendiri. Kebolehan minyak kastor dalam menyerap ethanol memberi impak yang besar dalam bidang ini. Oleh itu dalam projek ini Escherichia coli K011 merupakan sejenis bakteria digunakan untuk proses menukar glukosa kepada produknya iaitu ethanol. Akan tetapi kehadiran minyak kastor dalam proses fermentasi memberi impak kepada pertumbuhan dan proses pemakanan Escherichia coli K011 terhadap glukosa. Hal ini berlaku disebabkan beberapa perkara. Perkara yang pertama adalah ciri-ciri toksik yang ada pada minyak kastor memberi impak yang negative kepada Escherichia coli K011. Yang kedua pula adalah glukosa bukanlah substrate yang terbaik untuk Escherichia coli K011 untuk digunakan. Oleh sebab itu Untuk penghasilan ethanol yang lebih banyak xylose lebih sesuai digunakan.

Kata Kunci: *Bioethanol, Minyak mentah, Bahan buangan Tumbuhan, Fermentasi, Minyak kastor, Escherichia coli K011, glukosa dan xylose.*

CHAPTER 1

INTRODUCTION

1.1 Introduction

It is common knowledge that our main source of oil is decreasing as the result of increasing use in vehicular fuel. Therefore, researchers are making various efforts to find other fuel alternative to resolve this issue. One solution is the production of bioethanol from lignocellulosic waste. Thus, there is a very big opportunity in developing fuel ethanol from agricultural residue and other lignocellulosic feedstock because it is low in cost (Vincent *et al.*, 2011).

Currently, fermentation is the most used and preferred ethanol producing method (Vincent *et al.*, 2011). Although fermentation is the main process, there is a big problem with ethanol accumulation in the fermentation broth. Therefore, a solution is needed to solve the issue on the increasing amount of ethanol production in the fermentation broth. One possible way to overcome this obstacle is to use a selective separator such as ricinoleic acid, found in castor oil. In this study, we performed anaerobic fermentation using *Escherichia coli* K011 to convert glucose to ethanol. Castor oil was then added to assist in the selective separation of ethanol from the fermentation broth to maintain low concentration of ethanol in the fermentation vessel. The result of our experiment will have huge impact on the improvement of anaerobic fermentation production of ethanol.

1.2 Objectives

The objectives of this study are:

1. To study the effects of castor oil on *E. coli* K011 growth during anaerobic fermentation.
2. To determine the effect of castor oil on the fermenting activities of *E. coli* K011 during anaerobic fermentation.
3. To determine the optimum glucose concentration for effective ethanol production during anaerobic fermentation by *E. coli* K011.

CHAPTER 2

LITERATURE REVIEW

2.1 Castor Oil

Castor oil is an unedible plant oil (de Lima da silva *et al.,* 2009). It is a viscous, pale yellow non-volatile and non-drying oil with a bland taste and usually used as purgative (Oguniyi 2005). According to Oguniyi (2005), castor oil is produced from the extraction of castor seed (*Ricinus communis*). This plant seed contains high percentage of oil, which are about 46% to 55% of the total weight (Oguniyi, 2005).

Castor oil consists mostly (90% wt) of triglycerides 12-hydroxy-9-octadecenoic ester or ricinoleic acid. This fatty acid harbor a group hydroxyl group at C-12 that confers castor oil its unique chemical and physical properties (de Lima da silva *et al.,* 2009). Due to the present of the hydroxyl group castor oil is soluble in alcohol at room temperature 27 °C to 33 °C. Hence, this characteristic may be beneficial in the production of ethanol. Other than that, castor oil also dissolves easily in glacial acetic acids, chloroform, carbon sulfide and benzene (de lima da silva *et al.*, 2009). More characteristics of castor oil are shown in Table 1.

Table 1: Characteristics of castor oil (Oguniyi, 2005)

Properties	Cold- pressured oil	Solvent extracted oil	Dehydrated oil
Specific gravity	0.961–0.963	0.957–0.963	0.926–0.937
Acid value	3	10	6
Iodine value	82–88	80–88	125–145
Saponification value	179–185	177–182	185–188

$$\begin{array}{l} CH_2\text{-}O\text{-}C(O)\text{-}R \\ CH\ \text{-}O\ \text{-}C(O)\text{-}R \\ CH_2\text{-}O\text{-}C(O)\text{-}R \end{array} \quad + \quad \begin{array}{l} CH_2\text{-}O\text{-}C(O)\text{-}R \\ CH\text{-}O\text{-}C(O)\text{-}R \\ CH_2\text{-}O\text{-}C(O)\text{-}R^1 \end{array}$$

$R=\text{-}(CH_2)_7\text{-}CH=CH\text{-}CH_2\text{-}CH(OH)\text{-}(CH_2)_5\text{-}CH_3$
R^1= other fatty acid derivatives

Figure 1: Composition of castor oil (Oguniyi, 2005)

In a study performed by Akaranta and Anusiem (1996), extraction was done by using solvent such as hexane to get the maximum oil content from castor seed (Figure 2).

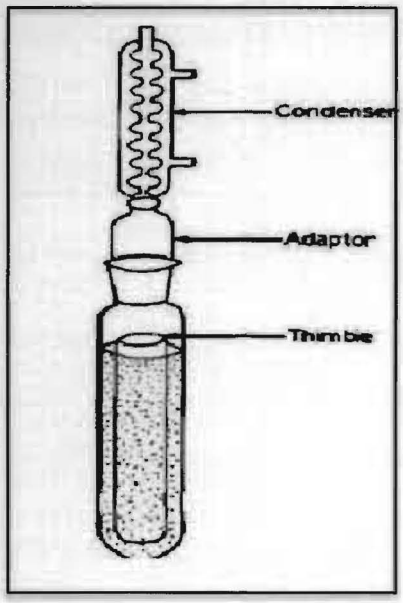

Figure 2: Castor seed oil extractor (Akaranta and Anusiem, 1996)

Hexane can be used it does not affect the structure of the fatty acids molecule in castor oil. The specific interaction of the hydroxyl and carboxylic acid groups of the fatty acid molecules with feint hydroxyl group can be further manipulated for the field of fermentation. In another study by Offerman *et al.* (2006), Castor oil, ricinoleyl alcohol and methyl ricinoleate was found to possess higher ethanol distribution coefficients and reduce separation factors (Offerman *et al.*, 2006). Therefore, castor oil has considerable affinity toward alcohol. Therefore, by adding castor oil in fermentation broth during ethanol production, ethanol will preferably be absorbed in the castor oil layer.

2.2 *Escherichia coli* K011

Escherichia coli is a bacterium in the family of *Enterobacteriaceae*. It is a rod-shaped organism with a circular genome of about 4500 kb in length (Chow *et al.*, 2009). This bacterium is easy to grow and prepare. According to Chow *et al.* (2009) there are two common ways to grow this bacterium, which are in solid and in liquid media. A liquid media that commonly used is Luria Broth (LB).

Presently, many strains have been developed and *E. coli* K011 is one that is frequently used in fermentation process beside *Klebsiella oxytoca* and *Zymomonas mobilis* (Walton, 2009). Walton (2009) stated that *E. coli* metabolize many sugar, has a very simple growth requirement and can be used in many industrial application. However, it tends to over accumulate acetic acid and succinics acid (Walton, 2009). This has led to the modification of this bacterium by the insertion the PET (production of ethanol) aperon into the pyruvate formate lyase gene of *E. coli*. This modification caused a change in the gene of *E. coli* that disrupts to the terminal fumarate reductase gene of the succinate pathway. This new strain was the called as *E. coli* K011 (Walton, 2009). Therefore, the development of *E. coli* K011 has led advancement to ethanol fermentation due the capability of this strain to actively metabolizing a variety of substrate including hexoses, pentoses and lactoses (Ingram *et al.*, 1987).

2.3 Anaerobic Fermentation of *E. coli* K011

Fermentation is the process of conversion of any polysaccharide or monosaccharide such as glucose to alcohol, carbon dioxide and organic acid by using yeast or bacteria which are conducted under anaerobic condition. Fermentation can also be referred to the glycolysis process in the absence of oxygen (Dekker *et al.,* 1988). According to Ingram *et al.* (1987), during the glycolysis under the absence of oxygen, mammalian tissue will produce more lactic acid, whereby plant will produce ethanol as the fermentation product. For the *E. coli* K011, without present of oxygen, pyruvate molecules will be reduced to ethanol with the release of carbon dioxide (Bai *et al.*, 2008). Anaerobic fermentation is very important in industrial operation because this process main source to produce bread and fermented beverages to the world (Dekker *et al.,* 1988).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The materials that used in this study were:

1. Castor oils
2. PBS buffer
   i. Sodium Chloride, NaCl (Merck, Germany)
   ii. Potassium Chloride KCl (J. T. Baker, USA)
   iii. Sodium Hydrophosphate, Na_2HPO_4 (Merck, Germany)
   iv. Potassium di-hydrogen phosphate KH_2PO_4 (J. T. Baker, USA)
2. *Escherichia coli* K011
3. Distilled water
4. D-(+)- Glucose (Sigma,USA)
5. Luria broth (Sigma,USA)
6. Plate Count Agar (Merck, Germany)
7. Fermentation Broth: (Dowe and McMillan, 2008)
   i. 10X YP medium (liquid)

-Yeast extract (CONDA, Spain)	100 g/L
-Peptone (CONDA, Spain)	200 g/L

3.2 Methods

3.2.1 *E. coli* K011 Preparation

E. coli K011 preparation is done in growing cultures within 100 ml of LB broth at 37 °C with constant agitation at 120 rpm. The culture is done overnight. Then the cell harvested through centrifugation in 50 ml conical centrifuge tubes (Vincent *et al.*, 2011).

3.2.2 Fermentation Broth

The fermentation broth is prepared by mixing of all the reagent and medium in 250 ml glass bottles. The fermentation broth was prepared by mixing the YP solution with pre-determined glucose weight and *E. coli* K011 as the fermenting agent. The glucose concentrations were set at 5%, 10% and 15% respectively. One set of control without castor oil was prepared (Figure 3). A second set was added with 10 ml of castor oil (Figure 4). The fermentation broths were then incubated for five day.

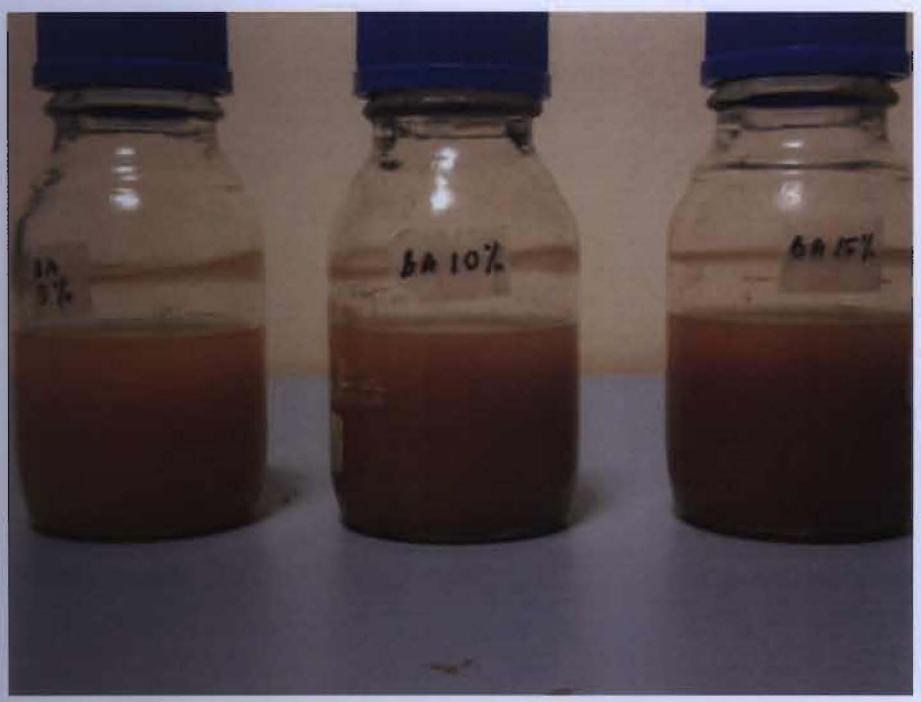

Figure 3: Photo showing that experiment conducted without castor oil in different concentration of glucose.

Figure 4: Photo showing that experiment conducted with castor oil in different concentration of glucose.

3.2.3 Solubility Test of Castor Oil

This procedure was done by using different percentages of water and ethanol.

Table 2: Mixture of solubility test

Castor Oil (ml)	Ethanol (%)	Water (%)
10	100	0
10	80	20
10	60	40
10	40	60
10	20	80
10	10	90
10	8	92
10	6	94
10	4	96
10	2	98
10	1	99

10 ml of various ethanol: water mixtures (Table 2) were prepared. Castor oil was then poured on top of mixture and place in an incubator shaker (120 rpm) at 37 °C overnight. After the incubation period, 1.5 ml sample were collected by pipetting the water phase solution mixture. Then, the samples were centrifuge to separate the castor oil and the water. The water phase was in in sent for HPLC analyses to determine the ethanol residues.

3.2.4 Viable Cell Count Analyses

This analysis is done under plate count agar. After preparation is done sample are taken and 300 µL is the amount that used in these analysis. Dilution factor that used for plate count mostly is 10^{-6} to 10^{-8}. According to Reynolds *et al.*, (2005), the most accurate colonies are 30 to 300 should be taken. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample),and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony-forming units (Reynolds *et al.*, 2005). Then after colonies are count from the plate (Figure 5) calculation is carried out from formula below.

$$\text{Colony forming units (CFUs)} = \frac{\text{Number of colonies}}{\text{dilution x amount plated}} \text{ Bacteria/mL}$$

Figure 5: Photo showing that *E. coli* K011 colonies growth in plate count Agar.